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**Original Research Article**

# Vitamin $B_{12}$ status among anaemic adolescents

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## ABSTRACT

**Background:** Nutritional anaemia are common health problems. Most studies are done regarding iron deficiency anaemias. There are limited data regarding vitamin $B_{12}$ deficiency anaemia especially in adolescents. Aims and objectives of the study were to find the prevalence of vitamin $B_{12}$ deficiency among anaemic adolescents and to study various socio-demographic factors and hematological parameters associated with vitamin $B_{12}$ deficiency.
**Methods:** Cross sectional hospital based observational study of 211 adolescents (10 -18 year) with anaemia. Socio demographic characteristics like age, sex, education of mother & patient, socio- economic class, dietary history were noted for each patient. CBC including RBC indices and serum estimation of vitamin $B_{12}$ level were done for each patient.
**Results:** Anaemia was seen in 46.6% of adolescents. Vitamin $B_{12}$ deficiency was seen in 49.76%. Vitamin $B_{12}$ deficiency was significantly associated with male gender (p=0.032) and vegetarians (p=0.047). Moderate to severe degree of anaemia (p=0.016), macrocytosis (p= 0.000) and thrombocytopenia (p=0.007) was more observed in vitamin $B_{12}$ deficient patients as compared to vitamin $B_{12}$ non deficient groups. Statistically significant fall in mean haemoglobin level and increase in mean MCV values were seen with decreasing serum vitamin $B_{12}$ level.
**Conclusions:** Among anemic male and vegetarian adolescents vitamin $B_{12}$ deficiency is a significant health problem. Hematological findings in peripheral blood are more associated with severe degree of vitamin $B_{12}$ deficiency. Vitamin $B_{12}$ supplementation along with IFA should be addressed through national programmes.

**Keywords:** Adolescent, Anaemia, Hematological changes, Sociodemographic factor, Vitamin $B_{12}$ deficiency

## INTRODUCTION

Nutritional anaemia is one of the most important health problem throughout the world especially in developing countries due to its high prevalence in these regions.[1,2] Preschool children, pregnant women & adolescents constitute vulnerable group of anaemia.[3] Adolescence is a period of transition between childhood & adulthood, is second to infancy as the period of most rapid growth. During this period with inadequate & improper dietary habits, one is vulnerable to all kinds of nutritional morbidities.[1] The prevalence of anaemia among adolescents is 27% in developing countries and 6% in developed countries. In India, the prevalence in adolescent population ranges from 32-55%.[4]

Among nutritional anaemias, iron deficiency is the most common cause and it is a major public health concern for researchers and policy makers.[5] In addition to iron, adequate amount of other nutrients like folic acid and vitamin $B_{12}$ is required for haematopoiesis, deficiency of which leads to megaloblastic anaemia.[5,6] Vitamin $B_{12}$ deficiency is a reversible cause of bone marrow failure and demyelination of nervous tissue for which early recognition and treatment is critical.[7] Low levels of vitamin $B_{12}$ are less commonly associated with infertility

and thrombosis.[7] It can be a cause of recurrent abortion or preterm delivery in pregnant mothers.[5,8] Infants born to deficient mothers are at high risk for developing $B_{12}$ deficiency and irreversible neurological damage.[9]

Most of the studies related to anaemia were focused on iron deficiency anaemia and study population targeted was mainly adolescent females, pregnant mothers or young children. There is paucity of data regarding $B_{12}$ deficiency among adolescent population. This study was done to find the prevalence of vitamin $B_{12}$ deficiency among anaemic adolescents and to study various socio-demographic factors and hematological parameters associated with it.

## METHODS

A hospital based cross sectional observational study done at SMIMER hospital, Surat, India over a period of 1 ½ year, after approval by institutional ethical committee. The study population comprised of adolescent patients (10-18 year). Informed written consent was obtained from legal representatives of patients.

The criterion of anaemia was accepted as the haemoglobin (Hb) value below 12 g/dl. All the adolescent patients with Hb <12 g/dl were included in study. Patients having acute critical illness, chronic systemic disease, diagnosed cases of hemolytic anaemia, blood transfusion history in past, reticulocyte count >2% or patients unwilling for inclusion in study, were excluded from the study. A detailed history including socio demographic determinants like patient's age, sex, literacy level; maternal literacy level; socioeconomic status (SES) based on modified Prasad's classification and dietary history were recorded. Those consuming animal food like meat, fish at-least once per week were considered as non-vegetarians. Age group was divided as early adolescent (10-<13 year), mid adolescent (13 - <16 year) and late adolescent (16-18 year). Anthropometric measurements (weight, height) were noted. Body Mass Index (BMI) was categorised as under-nutrition (BMI <18.5 kg/m$^2$), normal (BMI 18.5-24.9 kg/m$^2$), overweight (BMI 25.0-29.9 kg/m$^2$) and obese (BMI >30 kg/m$^2$).

Hematological parameters like hemoglobin (Hb), total WBC count and platelet count, RBC indices MCV, MCH and MCHC were measured in venous blood by automated cell counter.

Reticulocyte count was done for all patients. Serum vitamin $B_{12}$ level estimation was done in venous blood using chemiluminescent enzyme immunoassay technique.

Hb level between 10-11.9 mg% was taken as mild anaemia, between 7-9.9 mg% moderate and <7 gm% as severe anaemia. Platelet count <1.5 lac/cmm was taken as thrombocytopenia. Based on MCV value patients were categorised as having microcytic (<80fl), normocytic (between 80 fl-100 fl) and macrocytic anaemia (>100 fl).

Vitamin $B_{12}$ level below 160 pg/ml were accepted as deficiency level as per laboratory cut off value.

Patients were grouped in two: group A- vitamin $B_{12}$ <160 pg/ml, group B-vitamin $B_{12}$ >160 pg/ml. For further analysis group A patients were divided in 3 subgroups; <60 pg/ml, 60-100 pg/ml, 100-160 pg/ml.

For statistical analysis of qualitative data Chi square test and quantitative data independent't' test and Annova test were applied.

## RESULTS

A total of 757 adolescent patients were admitted during study period, out of which 350 had Hb <12 gm%. Hence, prevalence of anaemia in our study was 46.6%. Out of 350 anemic patients, 13 patients took DAMA, 19 patients were critically ill and in 107 patients vitamin $B_{12}$ estimation were not done due to cost restraint. Finally 211 patients were subjected to statistical analysis. Out of 211 patients, 105 patient had vitamin $B_{12}$ level <160 pg/ml. Thus prevalence of vitamin $B_{12}$ deficiency in our study was 49.76%.

In Table 1, mean age of group A was 14.12 years and group B was 14.28 years. Maximum numbers of patients in both groups were found in 13-16 years (mid adolescent). Maximum patients belonged to lower middle class (41.90% vs. 48.11%). Maximum mothers were illiterate (47.61% vs. 41.50%). Most patients were educated up-to primary level (52.38% vs. 55.66%).Under-nutrition was seen in 69.25% patients of group A and 65.71% of group B. Statistical significance between vitamin $B_{12}$ level and age of patient (p=0.1023), socio-economic class (p=0.25), education of mother (p=0.66), education of patient (p=0.89) and BMI (p=0.9169) was not found.

Group A had 60 (57.14%) males as compared to 45 (42.45%) in group B. Male: Female ratio noted was 1.3: 1. Boys were more affected than girls with vitamin $B_{12}$ deficiency (p=0.032).

In group A, 80 (76.19%) patients were vegetarian and 26 (24.76%) were non-vegetarian. Statistical significance has been noted with regard to dietary habits between both groups (p=0.047), suggesting that vegetarians were more affected by vitamin $B_{12}$ deficiency.

In Table 2, Hematological parameters showed none of the patients with low total WBC count. In group A patients, 58(55%) had moderate anaemia, 34 (32%) had mild anaemia and 13 (12.38%) had severe anaemia as compared to 39.62%, 51.86% and 8.4% having moderate, mild and severe anaemia respectively in group B. Statistical significance was noted with regard to severity of anaemia in both groups, suggesting that vitamin $B_{12}$ deficiency was more seen with moderate to severe anaemia (p=0.016).

**Table 1: Characteristic of patients with anaemia (N=211).**

| Socio demographic variables | Group A (n=105) | Group B (n=106) | p value |
|---|---|---|---|
| **Age groups (in years)** | | | |
| 10-13 | 22(20.95%) | 36(33.96%) | 0.1023 |
| 13-16 | 54(51.42%) | 44(41.50%) | |
| 16-18 | 29(27.61%) | 26(27.35%) | |
| Mean age | 14.12 | 14.28 | |
| **Sex** | | | |
| Male | 60(57.14%) | 45(42.45%) | 0.03286 |
| Female | 45(42.85%) | 61(57.54%) | |
| **Socio economic class** | | | |
| Upper | 1(0.95%) | 1(0.94%) | |
| Upper middle | 37(35.23%) | 42(39.62%) | 0.2527 |
| Lower middle | 44(41.90%) | 51(48.11%) | |
| Upper lower | 23(21.90%) | 12(11.32%) | |
| **BMI** | | | |
| <18.5 | 73 (69.52%) | 44 (41.50%) | |
| 18.5 – 25 | 36 (34.28%) | 40 (37.73%) | 0.9169 |
| >25 | - | - | |
| **Education of mother** | | | |
| Illiterate | 50(47.61%) | 44(41.50%) | 0.668 |
| Primary | 35(33.33%) | 40(37.73%) | |
| Secondary | 20(19.04%) | 22(20.75%) | |
| **Education of patient** | | | |
| Illiterate | 2 | 2 | 0.8903 |
| Primary | 55(52.38%) | 59(55.66%) | |
| Secondary | 48(45.71%) | 45(42.45%) | |
| **Diet** | | | |
| Vegetarian | 80 (76.195) | 66 (62.26%) | 0.047 |
| Mixed | 26 (24.76%) | 39 (36.79%) | |

**Table 2: Hematological characteristic.**

| | Group A (n=105) | Group B (n=106) | p value |
|---|---|---|---|
| **Severity of anaemia** | | | |
| Mild | 34 (32.38%) | 55 (51.86%) | 0.016 |
| Moderate | 58 (55.23%) | 42 (39.62%) | |
| Severe | 13 (12.38%) | 9 (8.4%) | |
| **MCV** | | | |
| <80 | 40 (38.09%) | 65 (61.32%) | 0.000 |
| 80 – 100 | 48 (45.71%) | 41 (38.67%) | |
| >100 | 17 (16.19%) | 00 | |
| **Platelet count** | | | |
| < 1.5 lac | 44 (41.95%) | 26 (24.52%) | 0.007 |
| >1.5 lac | 61(58.09%) | 80 (75.47%) | |

In group A, 44 (41.95%) had thrombocytopenia as compared to 26 (24.52%) in group B, which was statistically significant (p=0.007).

Based on MCV value >100fl, macrocytosis was seen in 17 patients in group 1. None of the patients in group 2 had macrocytosis. In group A, 48 (45.71%) had normocytic anaemia and 40 (38.09%) had microcytic anaemia, while in group B maximum number of patients had microcytic anaemia 65 (61.32%). Vitamin $B_{12}$ deficiency was more likely to be associated with macrocytosis (p=0.000).

In Table 3, Mean values of various parameters were compared with different levels of vitamin $B_{12,}$ which confirmed the earlier findings of age and BMI not related with vitamin $B_{12}$ level.

**Table 3: Comparison of vitamin $B_{12}$ level with mean values.**

| Variable | Vitamin B12 level (pg/ml) | | | | p value |
|---|---|---|---|---|---|
| | <60 | 60 -100 | 100 -160 | >160 | |
| | n=13 | n=36 | n=56 | n=106 | |
| **Mean age** | 13.77 ± 1.83 | 14.28 ± 2.17 | 14.32 ± 2.02 | 14.28 ± 2.15 | 0.8578 |
| **Mean BMI** | 15.97 ± 2.58 | 17.56 ± 2.40 | 17.20 ± 2.88 | 17.21 ± 2.99 | 0.3932 |
| **Mean Hb** | 6.55 ± 3.21 | 8.72 ± 2.62 | 9.36 ± 1.53 | 9.75 ± 1.65 | 4.1E-07 |
| **Mean platelet count** | 1,69,846.2 ± 1,35,877.3 | 2,21,459.5 ± 1,05,078.4 | 1,76,535.7 ± 91,068.4 | 2,22,358.5 ± 1,23,521.7 | 0.99918 |
| **Mean MCV** | 98.84 ± 13.90 | 78.75 ± 16.58 | 78.82 ± 13.83 | 73.33 ± 11.60 | < 0.000001 |

There was no statistical significant difference noted in mean platelet count and severity of vitamin $B_{12}$ deficiency. Patients with vitamin $B_{12}$ level of <60 pg/ml had severe degree of anaemia (mean haemoglobin level <7 gm/dl). Statistically significant fall in mean haemoglobin level was found with increasing severity of vitamin $B_{12}$ deficiency (p =0.000000413). We also observed that mean MCV values were higher with more severe vitamin $B_{12}$ deficiency (p <0.000001). Hematological changes like severe anaemia and macrocytosis were late findings, more likely to be seen with severely low vitamin $B_{12}$ levels.

## DISCUSSION

We found 46.66% prevalence of anaemia in our study. Studies in prevalence of anaemia from different states of rural India, reported high prevalence of anaemia from 46-98%.[1,10,11] However, most of the study has target population of adolescent girls. As per WHO classification prevalence rate of anaemia >40% is considered as severe health problem.[12] Considering this, adolescent group should be considered as risk group for anaemia and special attention should be given on the strengthening of existing "package" of services for adolescents.

Studies from India reported up to 36% of infants and 47% of adults to have low serum vitamin $B_{12}$ concentrations.[13,15] Studies from other countries have reported the prevalence of 50%, 41% (Turkish infant & adolescent), 40% (Latin Americans).[9,13,16-19] Our study also showed high prevalence of vitamin $B_{12}$ deficiency of 49.76% among anaemic adolescents.

In study done in Colombian school age children, low vitamin $B_{12}$ concentration was strongly associated with age >9 year, male gender, increased maternal parity status, the amount of money spent on food at home and lower SES.[13] However such association with SES or education level of mother or patient was not seen in Guatemalan and Venezuelan study.[13,20,21] We also did not find age, socio economic class, maternal education level or patient's education level to be associated with $B_{12}$ deficiency. We observed statistical significance between male gender and vitamin $B_{12}$ deficiency which is similar to observation made by Velez et al.[23] This male predominance could be due to higher requirement of micronutrients in males than females to sustain their rapid growth.

The source of vitamin $B_{12}$ is mainly from foods of animal origin and synthesis by microorganism in body.[7] Various studies have found association between vegetarianism and low vitamin $B_{12}$ level.[13,16,23] In our study also patient having vegetarian dietary habits were more affected with vitamin $B_{12}$ deficiency suggesting that diet has major influence on vitamin $B_{12}$ status of individual.

Various hematological findings in patient with Vitamin $B_{12}$ deficiency are anaemia, neutropenia, macrocytosis, macrocytic anaemia, thrombocytopenia or pancytopenia.[24] Ineffective DNA synthesis accounts for majority of hematological changes seen in vitamin $B_{12}$ deficiency.

In our study, in vitamin $B_{12}$ deficiency group 55.23% subjects had moderate anaemia and 12.38% had severe anaemia as compared to 39.62% & 8.4% subjects in vitamin $B_{12}$ non deficient group. Atay et al reported 13% adolescents and 26% infants and Andres et al reported 37% elderly patients having anaemia with vitamin $B_{12}$ deficiency.[9,25] Our study population was anaemic adolescents, so we had higher number of patients having anaemia. Statistical significant difference was noted between mean haemoglobin values of $B_{12}$ deficient as compared to non $B_{12}$ deficient subjects, with greater fall in haemoglobin seen with severity of $B_{12}$ deficiency.

Neutropenia was reported less frequently in vitamin $B_{12}$ deficiency.[9] In our study we did not find any patient having neutropenia. Thrombocytopenia was observed in 41.95% of subjects; however no difference in mean platelet count was seen in relation to different vitamin $B_{12}$ level. Andres et al reported 9.9% incidence of thrombocytopenia in elderly patients with $B_{12}$ deficiency.[25] Obeid et el reported 3% incidence of thrombocytopenia in his study, however he found elevated mean platelet volume in 54% of subjects with vitamin $B_{12}$ deficiency.[26]

Finding of macrocytes on peripheral smear or MCV value (>100fl) has been suggested as one of the indicator of vitamin $B_{12}$ deficiency. In our study, we found macrocytosis in only 17% of patients. Comparing the mean MCV values with different level of vitamin $B_{12}$ showed that chances of getting macrocytosis (MCV >100 fl) were more with severe degree of Vitamin $B_{12}$ deficiency.

In literature, macrocytosis in subjects with vitamin $B_{12}$ deficiency has been reported at different rates, 36%, 23% and16.1%.[27-29] The MCV levels maybe normal in the one third of patients with Vitamin $B_{12}$ Deficiency.[26,27] Iron deficiency anaemia complicates the condition by masking macrocytosis.[26] Also significant megaloblastosis could be seen in bone marrow of Vitamin $B_{12}$ deficient subject, hematological finding in peripheral blood is not so common, a finding making it difficult to suspect vitamin $B_{12}$ deficiency.[26]

## CONCLUSION

Vitamin $B_{12}$ deficiency is an important cause of nutritional anaemia in adolescents especially in males and vegetarian population. Hematological finding of macrocytosis is a late occurrence. Severe degree of anaemia was more seen with severe vitamin $B_{12}$ deficiency, thus measurement of vitamin $B_{12}$ level of children in regions with low consumption of animal foods should not await for occurrence of hematological findings. National program should address vitamin $B_{12}$ deficiency problem along with iron & folic acid supplementation.

### *Limitation*

This is a hospital based study. Study population was anaemic adolescents. Follow up after vitamin B12 therapy was not done so we could not document improvement in hematological parameters after treatment.

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